Effect of Solvent Extraction on the Nitrogen Compounds in Alfalfa Protein Concentrates

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Freezing curd prepared from alfalfa juice, untreated or washed in water, was extracted with 2-propanol. Protein and non-protein nitrogen, crude protein, and amino acids were determined in unextracted concentrate and in the two extracted concentrates to examine the effect of extraction on its content. Direct extraction of unwashed freezing curd with 2-propanol increased the protein content by 15% but did not affect the percentage of non-protein nitrogen, which accounted for 10% of the total nitrogen; washing in water before extraction with 2-propanol yielded a 19% increase in the protein content and lowered the percentage of non-protein nitrogen by half. The amino acid profiles were similar for all three concentrates. However, the effect of extraction and washing on the individual amino acids differed, the most notable effect being the substantial increase in methionine in the extracted concentrates. The amino acid scores calculated on the basis of the amino acid composition were indicative of high protein quality of the extracted concentrates.

Keywords: *Alfalfa; amino acids; nitrogen; protein*

INTRODUCTION

Alfalfa leaf protein concentrates (LPCs) have been regarded as a potential alternative to conventional sources of edible protein. LPC has been assessed as food for children (Kamalanathan *et al.*, 1969, 1970; Oke, 1971, 1973; Olatunbosum *et al.*, 1972; Kamalanathan and Devadas, 1975) and has been included in food formulations (Toosy and Shah, 1974; Meimbam *et al.*, 1982; Lencioni *et al.*, 1984, 1987, 1989; Barbeau and Kinsella, 1987).

To be suitable for human consumption, LPCs must be chlorophyll-free. Such concentrates can be prepared according to either of two different methods. One method is to separate the soluble white proteins from the insoluble colored protein matter, although this method presents the drawback of lower yields of white protein concentrate (de Fremery *et al.*, 1973; Fiorentini and Galoppini, 1983). A second method is to extract whole protein concentrates with organic solvents; this method produces higher yields, and the extracted concentrate may be stored for extended periods without the need for low temperature or exclusion of oxygen (Bray, 1977).

Freezing alfalfa juice produces a curd, called freezing curd, that contains 50% of the dry matter and 60% of the nitrogen present in the original juice. Extraction of the curd with 2-propanol yields concentrates with high protein contents, low levels of lipids and polyphenols, and color and texture similar to those of white protein concentrates (Hernández *et al.*, 1988a, 1991). If solvent extraction is to be employed in the preparation of chlorophyll-free protein concentrates, it is essential to know how this process affects the protein content and the amino acid composition of the resulting concentrates.

The objective of the present study was thus to determine the total, protein, and non-protein nitrogen contents and the amino acid composition of three concentrates prepared from alfalfa freezing curd and to ascertain the effect of solvent extraction and washing of the freezing curd with water on the composition of nitrogen compounds. The amino acid score and the essential to nonessential amino acid ratios were also calculated for the three concentrates as measures of concentrate protein quality.

MATERIALS AND METHODS

Concentrate Preparation. Preparation of the freezing curd has been described elsewhere (Hernández *et al.*, 1988a,b, 1991) (Figure 1). Briefly, alfalfa was harvested, pulped, and pressed. The juice was poured into small containers and frozen at −25 °C until use. Each sample was thawed at room temperature for 18 h before use, as needed. Freezing curd so formed was separated from the thawed juice by centrifugation, and some of the freezing curd was freeze-dried to produce freezing concentrate (FC). Extraction with 2-propanol was carried out in a Soxhlet apparatus at the boiling point of the solvent using either untreated curd immediately after preparation, yielding 2-propanol-extracted freezing concentrate (IFC), or curd that had been washed by centrifugation with distilled water, yielding water-washed 2-propanol-extracted freezing concentrate (WIFC). Following removal of the residual solvent, the extracted freezing concentrates were ready for analysis.

Total Nitrogen Determination. The total nitrogen was analyzed according to the Kjeldahl method with endpoint potentiometric at pH 4.6. An indicator solution of 0.01 g of methyl red, 0.02 g of bromothymol blue, and 0.06 g of bromocresol green in 100 mL of 70% ethanol (v/v) was used for endpoint control. The color of this solution changes at pH 4.6 (Griepink *et al.*, 1983). A factor of 6.25 was used for protein conversion.

Protein and Non-protein Nitrogen. Two different reagents, trichloroacetic acid (TCA) and copper sulfate, were used for protein precipitation for purposes of comparison. In the TCA method, 250 mg of concentrate was weighed out into a centrifuge tube; 50 mL of 25% TCA was added, and the

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0021-8561/95/1443-3065$09.00/0

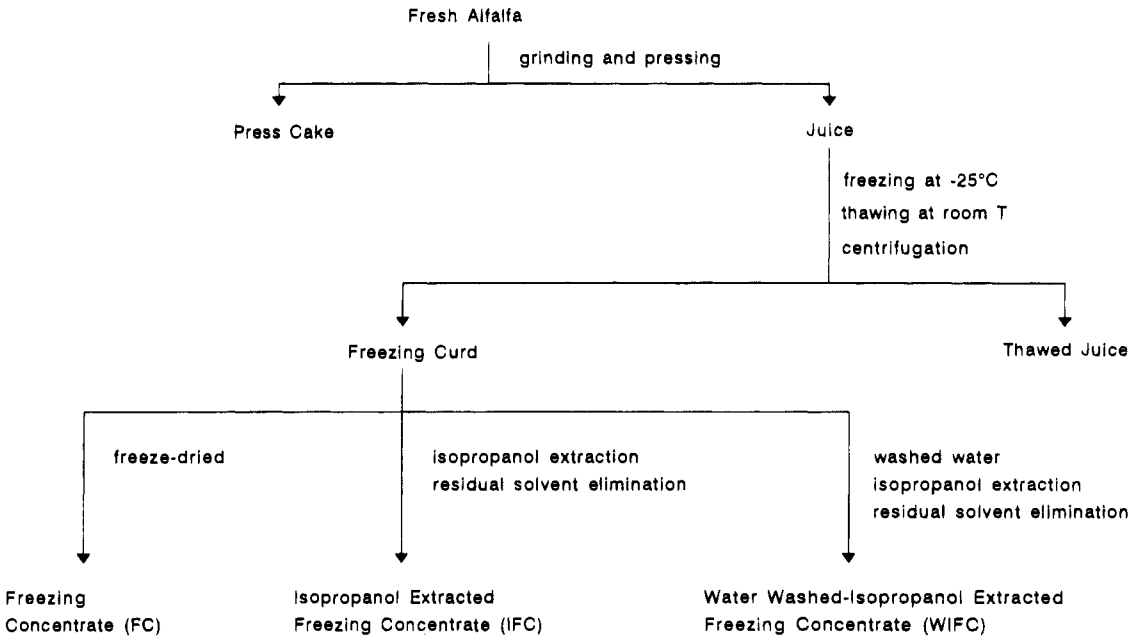

Figure 1. Obtention process of freezing protein concentrates.

mixture was shaken at 4 °C for 1 h, after which time it was centrifuged at 2500 rpm for 15 min. The residue was washed in 10 mL of 10% TCA at 4 °C for 15 min. All supernatant liquids were filtered into a Kjeldahl flask, and non-protein nitrogen was determined according to the Kjeldahl method.

In the copper sulfate method, 0.5 g of concentrate was weighed out into a precipitation tube; 50 mL of distilled water and two drops of silicone were added, and the mixture was boiled gently and shaken for 30 min. An amount of 2 mL of 10% aluminum–potassium sulfate and 50 mL of 3% copper sulfate was added hot, and the mixture was shaken until it had cooled to ambient temperature. It was then filtered through Whatman No. 54 paper and the residue washed in distilled water. All supernatant liquids were filtered into a Kjeldahl flask, and the non-protein nitrogen was determined according to the Kjeldahl method.

In both methods the protein nitrogen was calculated as the difference between the total and non-protein nitrogen.

Amino Acid Analysis. The amino acids were separated using a Beckman Model 600 autoanalyzer. Detection was carried out at 570 nm after postcolumn derivatization of the amino acids with ninhydrin. Three replicates of all analyses were performed.

The concentrate samples (100 mg) were hydrolyzed with 6 M HCl at 110 °C in a nitrogen atmosphere for 24 h. The proportion of HCl was 1 mL/mg of protein in the sample (Finley, 1985). The hydrolysate was filtered through Whatman No. 541 paper and the volume adjusted to 250 mL with Milli-Q water. An amount of 2 mL of solution was evaporated to dryness in a rotary evaporator at 40 °C. The hydrolysate residue was redissolved in 200 μL of 0.2 M citrate buffer (pH 2.2) containing norleucine as internal standard. An amount of 20 μL of this solution was diluted to 1000 μL with the said buffer, and 100 μL was injected into the autoanalyzer. Determinations of the amino acids cystine, proline, and tryptophan were not possible under the conditions of analysis employed.

Amino Acid Scores. Once the amino acid composition of the concentrates had been established, the essential to nonessential amino acid ratio and the amino acid score (AAS) were calculated for each concentrate. The essential to nonessential amino acid ratio was calculated by dividing the sum of the essential amino acids by the sum of the nonessential amino acids. The amino acid score was calculated using two recommended reference patterns, that proposed by FAO/WHO (1973) for adults and that proposed by the National Research Council (1980) for adolescents.

Table 1. Total Nitrogen (% DM) and Crude Protein Content (% N × 6.25) in the Alfalfa Protein Concentrates ($x \pm \sigma_{n-1}$, $n = 3$)

concentrate	% nitrogen	% crude protein
FC	8.35 ± 0.04	52.19
IFC	10.76 ± 0.01	67.25
WIFC	11.41 ± 0.04	71.31

RESULTS AND DISCUSSION

The total nitrogen and crude protein contents of the concentrates appear in Table 1. The crude protein content of the freezing concentrate was 52%. Literature reports indicate that the crude protein content of unfractionated concentrate obtained by heat treatment ranges between 50 and 60% (de Fremery *et al.*, 1973; Bickoff *et al.*, 1975; Gastineau and de Mathan, 1981; Hanczakowski *et al.*, 1991). Preparing the concentrate by coagulation in an acid medium followed by dialysis against water yielded a crude protein content of 59% (Wang and Kinsella, 1975).

The crude protein content of green concentrate obtained by coagulation under heating was 45–47% (Bickoff *et al.*, 1975; Edwards *et al.*, 1975; Gastineau and de Mathan, 1981; Hanczakowski *et al.*, 1991). Preparation of green concentrate using high molecular weight polyelectrolytes yielded a protein content of 43.5% (Baraniak and Baraniak, 1987), and preparation using organic solvents to precipitate the proteins in an acid medium yielded a protein content of 55% (Bray and Humphries, 1978a). These literature values suggest that the protein content of unfractionated concentrate is higher than that of green concentrate. The protein content is similar to that of unfractionated concentrate only when it is produced by precipitation with organic solvents.

Comparison with the aforesaid literature values shows the percentage protein content of the FC to be similar to those of unfractionated concentrate and green concentrate obtained by precipitation using organic solvents. This suggests that the FC contained soluble proteins in addition to cell wall proteins and is consistent with the hypothesis that it is composed mainly of chloroplasts (Hernández *et al.*, 1988a, 1989).

The protein contents of the IFC and WIFC were 67 and 71%, respectively. Accordingly, extraction with

Table 2. Protein (PN) and Non-protein Nitrogen (NPN) Content (% DM) in the Alfalfa Protein Concentrates As Determined According to the TCA and Copper Sulfate Methods ($x \pm \sigma_{n-1}$, $n = 3$)

	TCA		copper sulfate	
concentrate	% NPN	% PN	% NPN	% PN
FC	0.70 ± 0.06	7.64	0.68 ± 0.01	7.69
IFC	0.72 ± 0.01	10.04	0.72 ± 0.01	10.04
WIFC	0.34 ± 0.05	11.07	0.38 ± 0.01	11.03

2-propanol increased the protein content by 15%, and washing the curd with water before extraction yielded a further 4% increase. Bray *et al.* (1978b) considered the effect of extraction with polar and nonpolar solvents at ambient temperature on the green concentrate of alfalfa and reported that extraction with 2-propanol, ethanol, acetone, or butanol improved protein yields by 12%. Using two solvents in succession achieved an additional 5% increase. Hanczakowski *et al.* (1991) increased protein yields by 6% by washing alfalfa green concentrate in water and 5% by extraction using ethyl ether. Berot and Briffaud (1983) found that extracting rapeseed meals with alcohol/water mixtures (60:40 v/v) at ambient temperature raised protein contents by amounts ranging between 10% for methanol and 13% for 2-propanol.

Thus, the increased protein contents in the FCs prepared by extraction with 2-propanol were similar to the increases reported by Bray *et al.* (1978b) and Berot and Briffaud (1983) using polar solvents. The slightly higher increase (2–3%) recorded for the FC may be ascribable to the fact that the extraction procedure involved heating. The effect of washing in water on the percentage protein content of the FC was comparable to that obtained by Hanczakowski *et al.* (1991).

Table 2 shows the results of the protein and non-protein nitrogen determinations for the different concentrates by the TCA and the copper sulfate methods. Statistical analysis of the results indicated that there were no significant differences ($p < 0.05$) between the values obtained according to these two methods. It can therefore be concluded that the protein precipitation activities of the two reagents, TCA and copper sulfate, are comparable.

Table 2 also indicates that only 0.7% of the nitrogen in the FC was non-protein nitrogen. There are few literature values on the protein and non-protein nitrogen contents of alfalfa protein concentrates. Wang and Kinsella (1975) reported a non-protein nitrogen content of 0.49% in alfalfa protein concentrates obtained by acidification. However, the said workers dialyzed the concentrate before freeze-drying it.

There were no statistically significant differences ($p < 0.05$) between the non-protein nitrogen content of the FC and the IFC, but the non-protein nitrogen contents of the FC and the WIFC did differ significantly. Thus, while non-protein nitrogen was unaffected by extraction with 2-propanol, washing in water removed approximately 52.7% of the non-protein nitrogen. There are no literature values on the effect of extraction and washing in water on the non-protein nitrogen in protein concentrates.

The results indicate that less than 10% of the nitrogen in the FC and IFC was non-protein nitrogen and that washing in water before extraction, besides resulting in higher protein yields, also lowered the non-protein nitrogen by about half.

Table 3 presents the amino acid composition of the alfalfa concentrates; the values have been expressed as

Table 3. Amino Acid Composition (g/16 g of N) and Essential to Nonessential Amino Acid Ratio in the Alfalfa Protein Concentrates

amino acid	FC	IFC	WIFC
Ala	6.57	6.80	6.84
Arg	7.63	8.35	9.03
Asp	12.13	12.34	12.19
Glu	12.35	13.11	13.07
Gly	6.20	6.45	6.51
Ser	5.22	5.18	5.23
total NEAA	50.10	52.23	52.87
His	2.87	3.07	3.70
Ile	7.70	6.25	6.30
Leu	12.26	11.47	11.39
Lys	6.20	6.24	6.89
Met	2.54	3.23	3.14
Phe + Tyr	15.03	14.46	13.10
Thr	6.18	6.12	6.16
Val	6.26	7.38	7.46
total EAA	59.04	58.22	58.14
EAA/NEAA	1.18	1.11	1.10
%EAA	54.09	52.71	52.40

grams of amino acid per 16 g of N. Recovery of the nitrogen by the analysis was 93% in the FC, 95% in the IFC, and 97% in the WIFC.

The amino acid profile of the FC was similar to those of other alfalfa protein concentrates reported in the literature (Bickoff *et al.*, 1975; Wang and Kinsella, 1975; Gastineau and de Mathan, 1981; Terapuntuwat and Tasaki, 1986). The main amino acids present were aspartic acid, glutamic acid, leucine, isoleucine, phenylalanine, and arginine; histidine and methionine were the amino acids with the lowest concentrations.

Table 3 also shows that the amino acid profile was similar for all three concentrates, though the proportions of glutamic acid, valine, and arginine increased and the proportions of leucine, isoleucine, and phenylalanine decreased in the IFC. Washing the curd in water before extraction resulted in appreciable losses only in the amino acids tyrosine and phenylalanine, suggesting that a portion of those amino acids was present in free form or in the form of small peptides soluble in water during the washing stage. The WIFC exhibited high concentrations of the basic amino acids histidine, lysine, and arginine. These amino acids tend to be present in larger amounts in white protein concentrates than in green protein concentrates (Byers, 1971).

Because LPCs are characterized by their low methionine content, the high proportion of this amino acid in the extracted concentrates is notable. Literature values for this amino acid range from 1.8 to 2.7 g/16 g of N (Bickoff *et al.*, 1975; Wang and Kinsella, 1975; Gastineau and de Mathan, 1981; Hanczakowski *et al.*, 1991). The value for the FC was at the upper limit for that range. Wang and Kinsella (1975) pointed out that the content of this amino acid might depend upon the method used to obtain the concentrate, and they reported that the lowest methionine values were associated with concentrates produced by heating.

Table 3 also gives the ratio of essential to nonessential amino acids for the three concentrates. The ratio values were 1.18 for the FC and around 1.10 for the extracted concentrates. These ratios are in good agreement with the values calculated from literature values for other

Table 4. Amino Acid Scores (%) for the Alfalfa Protein Concentrates

concentrate	amino acid score: FAO/WHO	amino acid score: National Research Council	limiting amino acid
FC	73	98	Met + Cys
IFC	92	>100	Met + Cys
WIFC	90	>100	Met + Cys

Table 5. Amino Acid Score and First-Limiting Amino Acid in Various Foods (Krause and Mahan, 1984)

food	amino acid score	limiting amino acid
casein	91	sulfur
cow's milk	95	sulfur
whole egg	100 (126)	
rice	66	Lys
wheat	53	Lys
maize	49	Lys
soy flour	74	sulfur

alfalfa protein concentrates under the same conditions (Bickoff *et al.*, 1975; Wang and Kinsella, 1975; Gastineau and de Mathan, 1981; Terapuntuwat and Tasaki, 1986) as well as for leaf concentrates from other plant species (Byers, 1971). The ratio values calculated on the basis of the literature results ranged between 1.10 and 1.26. This corroborates Byers' (1971) finding that the amino acid compositions of LPCs were similar among different species.

Krause and Mahan (1984) reported that children and adolescents required more protein per kilogram of body weight than adults and that a higher proportion of those proteins should consist of essential amino acids. Scrimshaw (1976) recommended that the percentage contribution of essential amino acids to the total should be 43% in children, 36% in adolescents, and 19% in adults. Table 3 shows that 54% of the amino acids in the FC were essential amino acids and that approximately 52% were essential amino acids in the extracted concentrates, thus exceeding the recommended percentage for children in all three concentrates.

The amino acid scores for the three concentrates calculated on the basis of the amino acid reference patterns recommended by FAO/WHO (1973) and the National Research Council (1980) appear in Table 4. Only the FC was deficient in sulfur amino acids with respect to the National Research Council pattern, at a score of 98. With scores of 73, 92, and 90 for FC, IFC, and WIFC, respectively, all three concentrates were deficient in sulfur amino acids judged on the basis of the FAO/WHO pattern. However, two factors should be borne in mind: first, cystine could not be quantified, and consequently those amino acids have been underestimated; second, tests on adolescents carried out by Harper (1981) indicated that their sulfur amino acid requirements were closer to the recommendations of the National Research Council than to those of FAO/WHO. Although it was not possible to calculate the tryptophan score, the content of this amino acid in LPCs is known to be higher than that in recommended reference patterns (Bickoff *et al.*, 1975; Fiorentini and Galoppini, 1983).

Table 5 presents the amino acid scores and the first-limiting amino acids for various foods commonly consumed in human diets. Only whole egg is a complete protein source, and the amino acid scores for the extracted concentrates were similar to those for casein and somewhat lower than, though still of the same order of magnitude, as cow's milk, in which sulfur amino acids are also deficient. The amino acid composition and the proportion of the different amino acids in the extracted concentrates thus appear to be indicative of high protein quality, and the results therefore support the use of such concentrates as protein ingredients or as protein supplements in foods.

LITERATURE CITED

Baraniak, B.; Baraniak, A. Application of polyelectrolytes to fractionation of alfalfa juice protein. *Nahrung* **1987**, *31*, 341–343.

Barbeau, W. E.; Kinsella, J. E. Formation of a gel from a heated emulsion of alfalfa leaf protein and peanut oil. *J. Food Sci.* **1987**, *52*, 1030–1032.

Berot, S.; Briffaud, J. Parameters for obtaining concentrates from rapeseed and sunflower meal. *Qual. Plant Plant Foods Hum. Nutr.* **1983**, *33*, 237–242.

Bickoff, E. M.; Booth, A. N.; de Fremery, D.; Edwards, R. H.; Knuckles, B. E.; Miller, R. E.; Saunders, R. M.; Kohler, G. O. Nutritional evaluation of alfalfa protein concentrate. In *Protein Nutritional Quality of Foods and Feeds*; Friedman M., Ed.; Dekker: New York, 1975; Part 2, Vol. 2, Chapter 13.

Bray, W. J. The processing of leaf proteins to obtain food-grade products. In *Green Crop Fractionation*; Symposium 9; Wilkins, R. J., Ed.; British Grasslands Society: Hurley, 1977.

Bray, W. J.; Humphries, C. Solvent fractionation of leaf juice to prepare green and white protein products. *J. Sci. Food Agric.* **1978a**, *29*, 839–846.

Bray, W. J.; Humphries, C.; Ineritei, M. S. The use of solvents to decolorise leaf protein concentrates. *J. Sci. Food Agric.* **1978b**, *29*, 165–171.

Byers, M. The amino acid composition of some leaf protein preparation. In *Leaf Protein*; Pirie, N. W., Ed.; Blackwell: Oxford, U.K., 1971.

de Fremery, D.; Miller, R. E.; Edwards, R. H.; Knuckles, B. E.; Bickoff, E. M.; Kohler, G. O. Centrifugal separation of white and green fractions from alfalfa juice following controlled heating. *J. Agric. Food Chem.* **1973**, *25*, 886–889.

Edwards, R. H.; Miller, R. E.; de Fremery, D.; Knuckles, B. E.; Bickoff, E. M.; Kohler, G. O. Pilot plant production of an edible white fraction leaf protein concentrate from alfalfa. *J. Agric. Food Chem.* **1975**, *23*, 620–626.

FAO/WHO. Report of ad hoc expert committee on energy and protein requirements. Technical Report Series 522; WHO: Rome, 1973.

Finley, J. W. Reducing variability in amino acid analysis. In *Digestibility and Amino Acid Availability in Cereals and Oilseeds*; Finley, J. W., Hopkins, D. T., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1985.

Fiorentini, R.; Galoppini, C. The proteins from leaves. *Qual. Plant Plant Foods Hum. Nutr.* **1983**, *32*, 335–350.

Gastineau, I.; de Mathan, O. In *Proteines Foliares et Alimentation*; Costes C., Ed.; Gauthier-Villars: Paris, 1981.

Griepink, B.; Gonska, H.; Muntau, H. *BCR 63*; Community Bureau of Reference; Commission of the European Communities: Luxembourg, 1983.

Hanczakowski, P.; Szymczyk, B.; Skraba, B. Composition and nutritive value of native and modified green fraction of leaf protein from lucerne. *J. Sci. Food Agric.* **1991**, *56*, 495–501.

Harper, A. E. McCollum and directions in the evaluation of protein quality. *J. Agric. Food Chem.* **1981**, *29*, 429–435.

Hernández, A.; Martínez, C.; González, G. Freezing of alfalfa leaf juice. Formation and solvent extraction of freezing curd. *J. Sci. Food Agric.* **1988a**, *42*, 173–182.

Hernández, A.; Martínez, C.; González, G. Effects of freezing and pH of alfalfa leaf juice upon the recovery of chloroplastic protein concentrates. *J. Agric. Food Chem.* **1988b**, *36*, 139–143.

Hernández, A.; Martínez, C.; Alzueta, C. Effects of alfalfa leaf juice and chloroplast-free juice pH values and freezing upon

the recovery of white protein concentrate. *J. Agric. Food Chem.* **1989**, *37*, 28–31.
Hernández, T.; Hernández, A.; Martínez, C. Polyphenols in alfalfa leaf protein concentrates. *J. Agric. Food Chem.* **1991**, *39*, 1120–1122.
Kamalanathan, G.; Devadas, R. P. Leaf protein as supplement in preschool feeding programs. Presented at the 10th International Congress on Nutrition, Kyoto, 1975.
Kamalanathan, G.; Usha, M. S.; Devadas, R. P. Evolution of acceptability of some recipes with leaf protein concentrates. *Indian J. Nutr. Diet.* **1969**, *6*, 12–44.
Kamalanathan, G.; Nalinakshi, G. S.; Devadas, R. P. Effect of a blend of protein foods on the nutritional status of preschool children in a rural blawadi. *Indian J. Nutr. Diet.* **1970**, *7*, 288–291.
Krause, M. V.; Mahan, K. *Food, Nutrition, and Diet Therapy. A Textbook of Nutritional Cares*; Saunders: Philadelphia, 1984.
Lencioni, L.; Fiorentini, R.; Galoppini, C. *Ind. Aliment.* **1984**, *23*, 106–111.
Lencioni, L.; Pisanelli, A. M.; Baldi, E.; Fiorentini, R.; Galoppini, C. Sheep milk cheese made with the addition of alfalfa leaf protein concentrate. Proteolysis during ripening. *Lebensm. Wiss. Technol.* **1987**, *20*, 277–281.
Lencioni, L.; Pisanelli, A. M.; Baldi, E.; Fiorentini, R.; Galoppini, C. Sheep milk cheese made with the addition of alfalfa leaf protein concentrate. Acidity and texture during ripening. *Lebensm. Wiss. Technol.* **1989**, *22*, 81–87.
Meimbam, E. J.; Bautista, J. G.; Soriano, M. R. Studies on the fortification of biscuits with cassava leaf protein concentrates. *Philipp. J. Nutr.* **1982**, *35*, 82–86.
National Research Council (NRC). *Recommended Dietary Allowances*; National Research Council, National Academy of Sciences: Washington, DC, 1980.
Oke, O. L. Some aspects of leaf protein work in Nigeria. *Indian J. Nutr. Diet.* **1971**, *8*, 121–129.
Oke, O. L. Leaf protein research in Nigeria: a review. *Trop. Sci.* **1973**, *15*, 139.
Olatunbosum, D. A.; Adadevoch, E. K.; Oke, O. L. A new source for the management of protein calorie malnutrition in Nigeria. *Niger. Med. J.* **1972**, *2*, 195–199.
Scrimshaw, N. S. Strengths and weaknesses of the committee approach—an analysis of past and present recommended dietary allowances for protein in health and in disease. *New. Engl. J. Med.* **1976**, *294*, 136.
Terapuntuwat, S.; Tasaki, I. Effects of alcohol treatment and supplementation of methionine of corn oil on nutritive value of alfalfa leaf protein concentrate of young chicks. *Jpn. J. Zootech. Sci.* **1986**, *57*, 430–437.
Toosy, R. Z.; Shah, F. N. Leaf protein concentrate in human diet. *J. Sci. Ind. Res.* **1974**, *17*, 40–42.
Wang, J.; Kinsella, J. E. Composition of alfalfa leaf protein isolates. *J. Food Sci.* **1975**, *40*, 1156–1161.

Received for review December 28, 1994. Revised manuscript received June 13, 1995. Accepted August 29, 1995.[⊗]

JF940736D

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1995.